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14. ABSTRACT
High intensity focused ultrasound (HIFU) is a rapidly developing technique for cancer thermal ablation with secondary biological effects, such as heat shock response, that may be harnessed for adjunct therapies. To gain further insight into the potential for HIFU to be utilized in conjunction with gene therapy, a transparent cell-embedded tissue mimicking phantom was developed. Most physical parameters of the agrose-based hydrogel fell within the accepted range of values for soft tissues. Lesions produced under different combinations of HIFU intensity and exposure duration could be visualized inside the phantoms. GFP positive cells were primarily found within a circumferential region surrounding the primary site of lesion formation. The thermal necrosis boundary (EM43=240min) fell into this gene activation zone.

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HIFU, thermal ablation, heat shock response, gene activation

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INTRODUCTION:

The synergistic integration of high intensity focused ultrasound (HIFU) thermal ablation and HIFU-induced gene therapy represents a promising approach in improving the overall efficacy and quality of cancer therapy. Previous studies have demonstrated that HIFU can induce GFP gene activation under the control of hsp70B promoter in a murine tumor model. Thermal stress has been identified as the primary mechanism to regulate the gene expression. However, the natural heterogeneity and opacity of solid tumors has hindered direct correlation of site-specific gene expression level with *in situ* thermal dosimetry. We have developed a homogeneous and transparent cell-embedded tissue mimicking phantom as an alternative for simultaneous assessment of temperature distribution, HIFU lesion formation, and gene expression.

BODY:

Phantom Fabrication and Property Characterization

The phantom matrix is based on an agarose gel mixed with bovine serum albumin (BSA) and glycerol. Rat mammary carcinoma cells (R3230Ac) were stably transfected with green fluorescent protein (GFP) under the control of hsp70B promoter in advance. BSA, glycerol and the GFP transfected R3230Ac cells were added into the molten agarose/PBS solution at 35℃ before its setting. The resulting cell-imbedded tissue-mimicking phantom consists of 2% agarose, 3% (or 6%, 9%) BSA, 5% glycerol by weight, and the R3230Ac cells with concentration from $0.5x10^6$/ml to $5x10^6$/ml.

The speed of sound and attenuation were measured in a broadband transmission ultrasound (US) system with a through-transmission substitution technique. With or without the gel phantom, a broadband PVDF receiving needle hydrophone distinguished temperature-dependant arrival time and amplitude of the transmission signals from planar of 1~5 MHz range. The finite amplitude inset-substitution method with simplified modification was adapted to determine the second harmonic amplitudes with or without gel phantom. The corresponding ratio was used to determine the nonlinear parameter B/A under the monochromatic plane wave approximation. To fulfill this approximation, two planar transducers were aligned coaxially and placed 3 cm away facing each other, with the receiving transducer covered by a cork disk with a 3 mm pinhole in

the center. Thermal conductivity and diffusivity were measured with a thermal property analyzer. The compressive modulus of cylindrical gel phantoms was measured with a rheometer.

The speed of sound was found to be independent of BSA concentration in the range of 3% to 9%, while experiencing a steady rise from 1500 m/s at 20℃ to 1583 m/s at 70℃. The attenuation coefficient approximately linearly increased with rising ultrasound frequency from 1 MHz to 5 MHz for 6% BSA gels. The attenuation was also found to be temperature-dependent with a minimum at 40°C. The B/A value showed little variation within the BSA concentration range of 3% to 9%; it exhibited temperature-dependent, increasing from 5.5 at 20℃ to 6.3 at 70℃. Thermal conductivity and diffusivity were found to be independent of BSA concentration, but increased slightly from 20℃ (κ = 0.55 $Wm^{-1}℃^{-1}$, D= 0.13 mm^2s^{-1}) to 70℃ (κ = 0.64 $Wm^{-1}℃^{-1}$, D = 0.14 mm^2s^{-1}). The compressive modulus decreased continuously from 8.7 KPa to 4.7 KPa as BSA concentration was increased from 3% to 9%.

Lesion Formation and Gene Activation

Thermal lesions were produced in a gel phantom (with 6% BSA) at the focal point of a 3.3 MHz HIFU transducer (Figure 1). Temperature distribution was recorded by an embedded thermocouple array consisting of 5 bare-wire T-type thermocouples of 0.1 mm diameter. After HIFU treatment, the gel phantom was sectioned into ~1.5 mm thin slices along the direction parallel to the focal plane and cultured for another 24h before visualization of GFP activation by fluorescence microscopy.

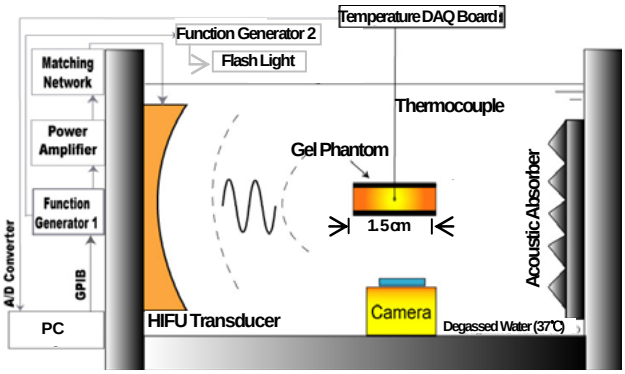

Fig. 1 SCHEMATIC DIAGRAM OF HIFU SYSTEM

Cigar or tadpole shape thermal lesions were observed inside the gel phantom, dependent on different combinations of ultrasound intensity and exposure duration. Typical lesion size ranged from 1x3 mm to 7.5x15 mm. One day following a 10s HIFU exposure, the white thermal lesion spot remained visible under phase contrast microscope. At this stage, GFP positive cells were primarily observed within a circular band in the focal plane. The *in situ* equivalent thermal dose EM_{43} in this plane was calculated based on the thermocouple array measurement. The correlated peak temperature for the gene activation zone after 10s HIFU exposure ranged from 54℃ to 63℃ (Figure 2). It is found that the empirical thermal necrosis boundary (EM_{43} =240min) fell into gene activation ring in both focal and beam planes.

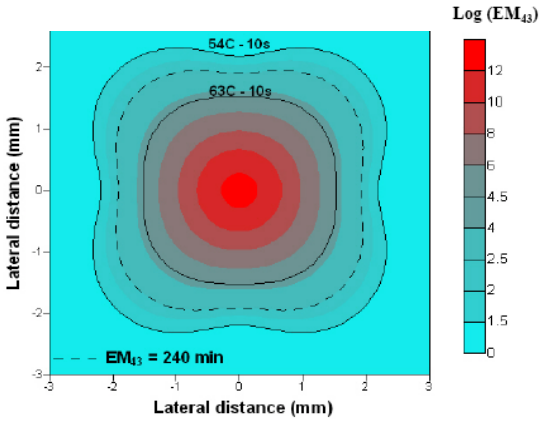

Fig.2 EM_{43} WITHIN FOCAL PLANE

However, to our surprise, the fluorescent yield of the cell line after HIFU treatment was found to be significantly lower than what we used to get before. We therefore performed hyperthermia test (30 min at 42℃) and eventually found out that the purity of cell line was significantly lower (Figure 3), which hindered us from achieving the expected gene activation after HIFU treatment. We consulted the issue with Dr. Chuanyuan Li who originally developed this cell line when he was at Duke University Medical Center. Two years ago, however, he had moved to a new position at the Medical Center of University of Colorado in Denver. Unfortunately, they didn't keep this cell line (which is not commercially available) during their relocation to Denver. Without other choice, we started to purify this cell line by repeated cell cloning.

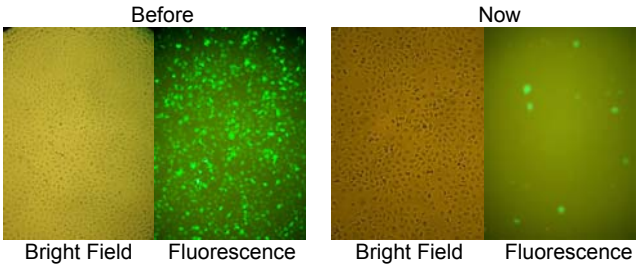

Fig. 3 Comparison of positive cells post hyperthermia

KEY RESEARCH ACCOMPLISHMENTS:

1. Reproduced and characterized cell-embedded gel phantom with improved protocol
2. Thermal dose distribution was analyzed and spatially correlated to gene activation zone

REPORTBAL OUTCOMES:

1. Yuan F, Pua C, Liu Y, Zhong P. HIFU-Induced Gene Activation in a Cell-Embedded Tissue Mimicking Phantom. *International Mechanical Engineering Congress and Exposition*, Seattle, WA, 2007

CONCLUSION:

We have developed a cell-embedded tissue mimicking phantom that has similar sound speed, acoustic impedance, thermal conductivity, thermal diffusivity and compressive modulus to the values in soft tissue, while the attenuation coefficient and nonlinear B/A ratio were comparably lower than those of soft tissue. The attenuation coefficient and the compressive modulus are the only parameters dependant on the BSA concentration. All the physical parameters are independent of cell concentration from $0.5x10^6$/ml to $5x10^6$/ml. The lesion geometry evolved gradually from axisymmetric cigar shape to non-axisymmetric tadpole shape as the total HIFU exposure increased. The development of tadpole shape lesion suggests vigorous cavitation and boiling within the focal region. The equivalent thermal dose calculation helped to correlate the gene expression pattern to *in situ* thermal dosage. The empirical thermal necrosis boundary was found located inside the gene activation ring, suggesting that gene activation was primarily induced in the sub-lethally injured cell population outside the HIFU lesion boundary. With purified cell line, future work is to establish a comprehensive gene activation pattern.

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